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Non-frozen transports of whole blood samples do not cause relevant bias for global coagulation tests in clinical trials evaluating the drug safety

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Abstract

Sample shipments with dry ice have a large economic impact on clinical research. Therefore, the bias caused for global coagulation tests by non-frozen transports of whole blood instead of frozen plasma was investigated experimentally and by a meta-analysis of 6-year central laboratory data. In the experiment, aliquots from 14 healthy volunteers were kept as whole blood at 20 ± 2 °C and as frozen plasma until an analysis of prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and antithrombin III (ATIII) at day 0, 1, 2, and 3 from collection. Within these 3 days only PT and aPTT demonstrated any changes: in blood samples kept at 20 ± 2 °C these amounted about 10% for both. In frozen plasma, aPTT did not change whereas PT increased by 14%. In a meta-analysis of central laboratory data, PT and aPTT results were grouped across various phase II–IV trials by the type of sample transfer, either as frozen plasma on dry ice or non-frozen as whole blood. For the latter the mean difference to a reference group of phase I trials with same-day analysis was in line with the amount of bias found in the experiment (aPTT, 34.6 ± 6.0 vs. 31.6 ± 3.5 s; PT, 87.7 ± 13.3 vs. 97.3 ± 7.9%). The consistent bias resulted in shifted, but still normal distribution curves with a total rate of clinically relevant outliers of about 1.9% for aPTT and 2.4% for PT. Biases thus appear irrelevant for a common safety evaluation within clinical trials. Non-frozen whole blood transports for the measurement of global coagulation tests appear justified

Abbreviations: ANOVA, Analysis of Variance; aPTT, Activated Partial Thromboplastin Time; AT III, Antithrombin III; BCT, Behring Coagulation Timer; FDA, Food and Drug Administration; INR, International Normalised Ratio; PT, Prothrombin Time; RILIBÄK, 'Richtlinie der Deutschen Bundesärztekammer'; SOP, Standard Operating Procedure; TT, Thrombin Time.

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for this purpose, if protocols do not require frozen shipments for other reasons. However, transit time must not exceed 2 days and pre-analytical conditions should be consistent within the same trial.

Keywords: Activated partial thromboplastin time; Antithrombin; INR; Prothrombin time; Stability; Thrombin time

1. Introduction

Coagulation parameters are generally considered to be rather unstable at room temperature and some to be activated by refrigeration [1,2]. Therefore an immediate analysis or freezing of samples is recommended [3,4]. On the other hand for most coagulation tests standardization of sample collection [5–9] and analytical methods [7,9–11] is still weak and results are hardly comparable across different laboratories. As a consequence, for clinical phase II–IV trials with patients and multiple investigators spread over various countries, samples are usually transferred as frozen plasma to one central laboratory for analysis. For clinical trials that aim to prove the drug safety only, this means significant extra costs, since the global coagulation tests are often the only reason for expensive shipments of samples on dry ice. Regarding hemostasis safety trials mostly include the activated partial thromboplastin time (aPTT) and/or the prothrombin time (PT), for which specifically in whole blood a stability for up to 24 h at room temperature has widely been accepted [3,8,10,12–17]. Accordingly, since 1998 our central laboratory has used non-frozen transportation of whole citrate blood for these tests, if clinical trials included them to monitor the drug safety only and participating investigators were in a zone of predicted 24 h transit time to the laboratory.

This study aimed to assess the bias caused by this pre-analytical procedure by (1) an experimental approach with healthy volunteers and (2) a meta-analysis of 6-year patient data from clinical trials. In the experiment the stability of PT, INR, aPTT, TT, and ATIII was tested in parallel in aliquots of frozen plasma and non-frozen whole blood for 3 days, resembling the maximum transit times to the lab as usual in international clinical trials. This period of time was not covered by most earlier experimental studies, nor was bias directly investigated by comparison of frozen and non-frozen conditions [7,8,10–15,18–21]. In a meta-analysis we compared means, ranges, and frequencies of clinically relevant outliers of PT and aPTT among phase II–IV trials in which either batch frozen plasma or non-frozen whole blood transportation was used. For the latter we also evaluated and compared distribution curves for samples that were received after 1, 2 or more than 2 days from collection. Phase I trials with a same-day analysis served as a reference. In combination, the experiment and the meta-analysis should enable an assessment of the relevance of the bias caused for global coagulation tests by non-frozen transport of citrate blood with regard to an evaluation of drug safety, the most common objective, for which these are measured in clinical phase II–IV trials.

2. Materials and methods

2.1. Experimental data

Four samples of citrate blood per individual were collected from 7 male and 7 female healthy volunteers between 23 and 59 years of age, who all gave written consent. Samples were collected in